Progress Report

Microorganism Study
J.P.L. Contract No. 950783

Sulfur Oxidizing Capacity of California Desert Soils

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Oregon State University
Corvallis, Oregon
March 9, 1967

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Sulfur oxidation in soils is chiefly due to specific oxidative activities of a few species of the bacterial genus Thiobacillus. Under favorable conditions these chemoautotrophic bacteria attack sulfides and sulfur, oxidizing them to sulfate as a source of energy. Because sulfate is an essential plant nutrient, these bacteria are important to soil fertility. They are present in all arable soils and are generally widely distributed wherever sulfides or sulfur is present. It is of interest to know whether or not sulfur bacteria are present in desert soils under severe environmental conditions. Rather than attempt their isolation and identification, which is difficult and time-consuming, their presence and activity can be determined by incubating a soil with added sulfur and subsequently determining the increase in sulfate. With this in mind, the following experiment was made with samples of six azonal California desert soils collected by Dr. Roy E. Cameron and submitted May 25, 1965 for sulfur oxidation studies.

A general description of the sites and soils has been given by Cameron (2). Additional characteristics, determined in the laboratory,

are shown in Table 1. One of the soils is slightly acid, one is neutral, and four are alkaline. The sulfur as sulfate is almost negligible, 1 to 3 ppm, in all the soils except 68-3, which showed 4440 ppm in the original sample. In general, humid soils are very low in sulfate while arid soils are often high.

Methods

After sieving through a 10-mesh screen and discarding the detritus, each soil was treated in the following manner. Four 50-gram portions, oven-dry basis, were spread onto paper squares. For controls two of these portions were transferred to pint milk bottles, 10 to 15 grams at a time with sufficient distilled water with each addition to satisfy 50 percent of the total water-holding capacity. Other than the incremental addition of the water, distributed as uniformly as possible from a pipet, the soil was not distrubed; shaking or mixing was avoided because it would cause compaction or puddling and interfere with normal aeration. The bottles were capped with DuPont polyethylene film, which is essentially impermeable to water vapor but allows adequate exchange of atmospheric gases. To each of the other two portions of soil, spread on the paper, was added 50 mg of flour sulfur. The sulfur was well mixed with the soil by use of a spatula and by rolling the mixture back and forth on the paper. The treated soil was then transferred to pint milk bottles, distilled water being added as described for the controls. All bottles were then placed in the incubator at 28°C.

After 30 days incubation all controls and sulfur treated soils were analyzed for pH and sulfate by the following procedure:

Sufficient distilled water was added to each bottle to make a 1:5

dilution. The bottles were then stoppered and mechanically shaken for 10 minutes. Approximately 50 ml of the suspension was transferred to a beaker for determination of pH, using a glass electrode apparatus equipped with a stirrer. Readings were made while the suspension was being stirred. After this, the supernatent from the beaker and the bottle was transferred to an Erlenmeyer flask and treated for clarification and determination of sulfate by the turbidimetric method (4).

Effective clarification was obtained with copper hydroxide. After addition of approximately 0.5 g cupric acetate and 0.3 g calcium hydroxide, the floc was allowed to settle and the solution was filtered through a Whatman No. 1 paper. Excess calcium was then removed by addition of ammonium carbonate. The calcium carbonate precipitate was filtered off and discarded, and the clear filtrate was used for sulfate determination.

To a portion of the cold filtrate acidified to litmus paper with HCl was added an excess of powdered crystalline $BaCl_2$. Sulfate was thus precipitated as colloidal $BaSO_4$. The resulting turbidity was determined by use of a Klett photometer and evaluated in ppm S as $SO_4^{=}$ by comparing readings with a standard curve. If the turbidity was too great for a reading a portion of the filtrate was first quantitatively diluted as required for an appropriate reading.

The results are presented in Table 2.

Discussion

Two of the soils, 76-2 and 196, oxidized none of the added sulfur during incubation and the pH changed little. All the sulfur was changed to sulfate in soil 68-3, but the pH dropped only 0.3, indicating a good buffer capacity. The 100 percent sulfur oxidizing capacity is unusual

but is occasionally found in arable soils (Table 3). The value of 116 percent (Table 6) may be attributable to errors inherent in the high dilutions required for suitable turbidimetric readings. However, the extensive and rapid oxidation of the 1000 ppm added sulfur indicates an active and efficient sulfur oxidizing microflora that, by attacking native oxidizable sulfur or sulfur compounds, could well account for the additional sulfate. The -0.3 percent shown for soil 76-2 is, on the other hand, due to unavoidable error in reading very low turbidity. The other three soils showed very low sulfur oxidizing power, lower than usually found in cultivated soils (Table 3).

From these results it may be concluded that soils 76-2 and 196 contain no sulfur oxidizing bacteria. Although present in the other soils, the sulfur bacteria in 68-3 were most active. Whether or not this was due to a more efficient strain of Thiobacillus in this soil, or due to more favorable soil properties is conjectural; the former seems more likely. That the incubated controls for all except 68-3 showed little increase over the original samples as received indicates a dearth of organic matter and/or sulfides in five of the soils.

Thiobacillus thiooxidans is probably the responsible organism in 9-2; this species, optimum pH 3, range 0.5-6.0, is active in acid soils only. In the other soils showing oxidation of sulfur the activity can be attributed mainly, if not entirely, to T. thioparus, which has an optimum pH of less than 7 and a range of 5.2-8.8. These optima and ranges have been determined with synthetic media; it is probable that the values would vary for different soils.

Data in Table 7 are representative of results obtained with a large number of soils by Bollen; many have been reported in several publications (1,3,4).

References

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Table 1

Pertinent Properties of Six California Desert Soils

Soil No.	Parent material	Texture	+ 10-mesh detritus		Water @ 105°C	Water Holding Capacity	pH	Sulfur as $SO_4^=$
			%		%	%		ppm
9-2	Quartz, plagioclase	Loamy sand, and stony loams	0.45	Stones to ½" grass	0.69	23.1	6.5	7
20	Quartz, plagioclase	Sandy, gravelly and stony loams	0.20	Pumice	0.00	26.3	7.0	7
51-3	Ancient beach sand	Indio very fine sand	3.38	Stones to ½" shells, pine needles	0.25	25.8	7.9	8
68-3	Extruded clay alluvium	Clay	0.10	Stones to ¼"	4.17	64.2	8.3	4440
76-2	Granite, sandstone	Sand	13.61	Stones to ½" fine roots	1.01	26.9	7.9	8
196	Cinders	Cindery	32.61	Stones to ¼"	0.00	31.2	8.0	8

Table 2

Sulfur Oxidizing Capacity of Six California Desert Soils

Soil No.	Sulfur as SO_4^{--}			Sulfur Oxidation	pH		
	Original	After 30 days incubation*			Original	After 30 days incubation*	
		control	with 1000 ppm S			control	with 1000 ppm S
	ppm	ppm	ppm	%			
9-2	3	10	39	2.9	6.5	5.6	5.2
20	2	16	38	2.2	7.0	6.9	6.9
51-3	1	7	42	3.5	7.9	8.4	8.0
68-3	4440	8585	9750	116.0	8.3	8.2	8.0
76-2	1	5	2	-0.3	7.9	8.0	7.5
196	2	0	0	0.0	8.0	8.2	7.7

* 30 days at 28°C with water at 50% of water-holding capacity

Table 3

Sulfur Oxidizing Capacity of Some Selected Arable Soils*

Soil	Location and Cover		Original pH	Sulfur Oxidation %
Altamont fine sandy loam	Oregon	alfalfa	6.2	11
Antelope clay adobe	Oregon	alfalfa	6.7	2
Case fine sandy loam	Oregon	alfalfa	6.4	9
Delhi sandy loam	California	fallow	6.8	11
Ephrata loamy fine sand	Oregon	nursery	7.8	56
Hood River silt loam	Oregon	orchard	6.8	46
Mazama pumice**	Oregon	barren	7.7	2
Newberry pumice**	Oregon	barren	7.5	0
Palouse silt loam	Oregon	virgin	6.8	85
Rattlesnake clay loam	Montana	pasture	6.5	50
Touchet clay loam	Washington	saline	7.3	16
Twairaj silt loam	Iraq	stubble	7.7	35
Umatilla medium sand	Oregon	alfalfa	7.2	28
Vale clay loam	Oregon	alkali	9.8	19***
Vale clay loam	Oregon	alfalfa	8.8	100***
Walla Walla clay loam	Oregon	virgin	6.9	39
Woodburn silt loam	Oregon	rye grass	5.6	66
Yolo clay loam	California	alfalfa	7.2	23

* Incubated 30 days with 1000 ppm flour sulfur at 28°C

** Non-arable

*** Incubated 115 days